Neospora caninum: Seroprevalence in beef cattle in the mountainous region of Santa Catarina, Brazil

Neospora caninum: Soroprevalência em bovinos de corte na região serrana de Santa Catarina, Brasil

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Abstract

The distribution of *Neospora caninum*, an important agent of reproductive disorders in cattle, has been frequently reported in dairy cattle in Brazil and worldwide. Records of infection are less frequent in beef cattle. To determine the seroprevalence of bovine neosporosis, 507 beef cattle blood samples were collected from January 2013 to September 2015, from 16 municipalities of the Associação dos Municípios da Região Serrana (AMURES). Samples were tested for the presence of IgG antibodies (≥1:100) against *N. caninum* by indirect immunofluorescence antibody test (IFAT). Information about sex, age, and origin of the bovines were obtained from the Brazilian Bovine and Bubaline Identification and Certification System (SISBOV), and were tabulated for statistical analysis (Chi-square and Fisher Exact test, $P \leq 0.05$). Of the 507 serum samples analyzed, 70 (13.81%) contained antibodies against *N. caninum* with titers of 1:100 (16), 1:200 (22), 1:400 (17), 1:800 (nine), 1:1600 (four) and 1:3200 (two). Positive specimens were collected from 13 of the 16 municipalities and across all age groups. Of the positive samples, there were no statistical differences between males and females (32.86% vs. 67.14%, $P=0.1072$), age groups ($P=0.4116$), or municipalities ($P=0.6838$). While not statistically significant ($P>0.05$), higher seroprevalence was observed among older females. Although seroprevalence was relatively low, results indicate that infection by *N. caninum* in beef cattle is widespread in the studied region.

Key words: *Neospora caninum*. Prevalence. Beef cattle. Santa Catarina.

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Resumo

A distribuição de *Neospora caninum*, importante agente de desordens reprodutivas em bovinos, tem sido frequentemente reportada em gado leiteiro no Brasil e no mundo, sendo menos frequentes os registros em bovinos de corte. Com o objetivo de determinar a soroprevalência da neosporose bovina, de Janeiro de 2013 a Setembro de 2015 foram colhidas 507 amostras de sangue de bovinos de corte, provenientes de 16 municípios integrantes da Associação dos Municípios da Região Serrana (AMURES), para a realização da Reação da Imunofluorescência Indireta (RIFI) para a detecção de anticorpos (≥1:100) IgG contra *N. caninum*. Informações de sexo, idade e procedência dos animais foram obtidas por meio dos registros no SISBOV (Serviço Brasileiro de Rastreabilidade da Cadeia Produtiva de Bovinos e Bubalinos), e foram tabulados para a análise estatística (Testes exato de Fisher e do Qui-Quadrado, P≤0,05). Das 507 amostras analisadas, 70 foram positivas, em 13 dos 16 municípios e em todas as faixas etárias foram identificados animais positivos. Os resultados indicam uma prevalência de 13,81% (70/507) com títulos de anticorpos de 1:100 (16), 1:200 (22), 1:400 (17), 1:800 (nove), 1:1600 (quatro) e 1:3200 (dois). Das amostras positivas 32,86% (23/70) foram de machos e 67,14% (47/70) de fêmeas, sem diferenças estatísticas (P=0,1072), assim como idade (P=0,4116) e município (P=0,6838). Embora sem correlação estatística (P>0,05), maior soroprevalência foi observada entre as fêmeas mais velhas. Apesar de a soroprevalência observada no presente estudo ter sido relativamente baixa, os resultados indicam que a infecção por *N. caninum* em bovinos de corte está amplamente distribuída na região estudada.

Palavras-chave: *Neospora caninum*. Prevalência. Bovinos de corte. Santa Catarina.

Introduction

In various regions of the world *Neospora caninum* is considered one of the main causes of abortions in dairy cattle (DUBEY, 1999; MARQUES et al., 2011) and can infect several animal species including domestic and wild canids, horses, goats and deers (DUBEY, 2003; VARASCHIN et al., 2012). In cattle, *N. caninum* has an efficient vertical transmission capacity (DUBEY, 2003), infecting 90% of animals in a herd (DUBEY et al., 2006) via transplacental, either exogenous or endogenous route.

It can also be transmitted horizontally to cattle by ingestion of sporulated oocysts excreted by infected dogs (DUBEY; SCHARES, 2011). Currently dogs (*Canis lupus familiaris*), coyotes (*Canis latrans*), dingoes (*Canis lupus dingo*) and gray wolves (*Canis lupus*), are the only species recognized as definitive hosts (DUBEY et al., 2011; GONDIM et al., 2004; KING et al., 2010; McALLISTER et al., 1998). It has been reported that the presence of coyotes may increase infection rates up to 2.4 times (CONZUELO SIERRA et al., 2011) and dogs up to 3.21 times (PORTOCARRERO et al., 2015), thus increasing the risk of infection in herd animals.

The primary sign observed in cows infected with *N. caninum* is abortion, usually between the fifth and seventh month of gestation (CAMPERO et al., 2003). Repeated estrus, successive miscarriages, and temporary anestrus are related to infection by *N. caninum* (BRUHN et al., 2013).

Reichel et al. (2013) estimated global losses caused by *N. caninum* to be more than $ 1 billion, with two-thirds of losses occurring in the dairy industry and one-third in the beef industry. In Brazil, *N. caninum* is widespread (CAMILLO et al., 2010), but little is known about its costs to the economy. Although rates of infection have been previously documented in this territory, it is believed that these exceed other abortive diseases such as brucellosis and leptospirosis (SARTOR et al., 2003). Reichel et al. (2013) estimated that in South America the annual cost associated with neosporosis can reach US $ 239.7 million per year, US $ 51.3 and US $ 101.0 million in the Brazil's dairy and meat industries, respectively.

Serological analysis of *N. caninum* associated with the occurrence of reproductive problems is an important tool to define the possible diagnosis and

to control outbreaks of the parasite in bovine herds (PAZ et al., 2007). The seroepidemiological results in Brazil have been variable. This is perhaps due to the number of samples collected, characteristics and size of herds, and lack of reproductive history of analyzed samples (CAMILLO et al., 2010).

There are several recent studies of *N. caninum*, both globally and in Brazil, however, little is known about the occurrence and prevalence of infection in beef cattle in the state of Santa Catarina, especially in the mountainous region, which is an important area for cattle reproduction. The objective of this study was to determine seroprevalence of *N. caninum* infection in beef cattle in the mountainous region of Santa Catarina, southern Brazil.

Material and Methods

From January 2013 to September 2015, 507 beef cattle blood samples were collected from 16 municipalities of the Associação dos Municípios da Região Serrana (AMURES). This mountainous region of Santa Catarina has an average altitude of 740 meters above sea level, with a minimum of 716 meters recorded in Abdon Batista and a maximum of 1353 meters in São Joaquin. The climate is a humid mesothermal type, with an average temperature of 16°C. There are also areas that experience subtropical and temperate climates with cold winters and temperatures reaching below 0°C.

The bovine population in the mountainous region of Santa Catarina is 557,826 (IBGE, 2012), representing approximately 18% of beef production in the state of Santa Catarina. The number of samples required to estimate the prevalence of *N. caninum* was calculated using an expected prevalence of 15%, 3.0% error and 95% confidence level (OPS, 1979).

The samples were taken at random from animals on properties in AMURES region and from four slaughterhouses, located in the municipalities of Lages (S.I.E 004), Otacílio Costa (S.I.E 620), São João do Itaperiú (S.I.E 041) and São Joaquim (S.I.E 470). Sera were stored (-20 °C) until processed for the detection of IgG antibodies against *N. caninum* by indirect immunofluorescence antibody test (IFAT).

Of the 507 samples, 215 (42.41%) were males and 292 (57.59%) females. The animals were also divided by age, establishing four groups: $0 \leq a \leq 2$ years (n=68, 13.41%), $2 < a \leq 4$ years (n=195, 38.46%), $4 < a \leq 8$ years (n=139, 27.42%) and >8 years (n=105, 20.71%).

To perform the IFAT, according to Dubey et al. (1988), NC1 tachyzoites of *N. caninum* strain were used as the antigen. Samples that showed a reaction at the dilution 1:100 were considered positive (MINERVINO et al., 2008) and were sequentially diluted in multiples of two, to reach the final reactive maximum dilution for titration. For comparison, positive and negative sera were used as controls.

Information about sex, age, and origin of the animals were obtained through the records in the Brazilian Bovine and Bubaline Identification and Certification System (SISBOV), and were tabulated for statistical analysis (Chi-square and Fisher Exact test, $P \leq 0,05$) to correlate the results of the serological analysis with the population data.

This study was approved by the Animal Experimentation Ethics Committee (CETEA), of the Agroveterinárias Science Center (CAV) of the State University of Santa Catarina (UDESC) under protocol 01.29.14, of June 3, 2014.

Results and Discussion

Of the 507 serum samples analyzed, 70 had antibodies against *N. caninum*, resulting in a prevalence of 13.81% in the mountainous region of Santa Catarina. These results are similar to those recorded in a study by Moura et al. (2012a), which found a prevalence of 13.2% in beef cattle in the micro region of Guarapuava, state of Paraná, Brazil.

Positive animals were identified in 13 of the 16 municipalities and across all age groups. Twenty-three (32.86 %) of the positive samples were males and 47 (67.14%) female. The antibody titers and number of positive animals were 1: 100 (16), 1: 200 (22), 1: 400 (17), 1: 800 (nine), 1: 1600 (four) and 1: 3200 (two).

Previous studies have shown the circulation of the agent in different species in the mountainous region of Santa Catarina with occurrence of IgG antibodies against *N. caninum* in 13.0% of dogs in urban areas of Lages (MOURA et al., 2011), 21.2% of dogs in rural areas of Lages (MOURA et al., 2012b), 2.6% of horses (MOURA et al., 2013), 4.58% of goats (TOPAZIO et al., 2014) and 5.83% and 7.0% of sheep (DALLA ROSA et al., 2011; MOURA et al., 2014).

In a comparable study conducted in the mountainous region of Santa Catarina, Moura et al. (2012b) reported seropositivity (IFAT, ≥1: 200) in dairy cattle (23.1%) to be higher than that observed in beef cattle from this study (13.81%). In addition to the different cutoff points used (1:200 vs. 1:100), this discrepancy of results, in the same animal species and in the same region, may be linked to the different management conditions employed in each production system (beef or dairy) (DUBEY et al., 2007), or to differences in the mechanism of the humoral response of dairy and beef (SANTOLARIA et al., 2011). For example, the extensive management beef cattle are subjected to in this region does not favor their contact with the dogs. The opposite occurs with dairy cattle, which are herded at least once a day to the milking facilities near the headquarters, where normally focus the dogs of the properties. In addition, beef cattle in the region studied rarely receive supplementation, which can also be related to lower prevalence in these animals. In comparison, dairy cattle receive concentrate, which has been identified as a risk factor for infection by *N. caninum* in bovine (ARREOLA-CAMBEROS et al., 2012). In contrast, Hasegawa et al. (2004) reported that winter supplementation

(corn, silage, sugarcane and Napier) did not affect the infection rate for *N. caninum* in beef cattle in the region of Avaré (SP).

Also, with respect to the type of animal (beef or dairy), Eiras et al. (2011), observed a greater number of dairy herds containing at least one positive animal, when compared to beef herds in Spain. However, individually, beef cattle, presented higher seropositivity. In comparison, Vanleeuwen et al. (2006) observed a higher seroprevalence of neosporosis in cattle beef herds than in dairy herds in Canada, but no difference was observed in the case of individual animals. These authors concluded that these differences may be partly due to employee tests to laboratories where they performed the tests or both, which can make inappropriate statistical comparisons in certain situations. These findings show variable results and indicate that only the type of activity does not to influences decisively the prevalence of infection in cattle.

This study found no significant differences in seropositivity between the municipalities (P> 0.05). Geographic differences in the distribution of animals infected by *N. caninum* have been reported in other studies and may be related, among other factors, to the relative abundance of the definitive host (canidae) as well as the management systems utilized (NASIR et al., 2012).

There were no statistically significant differences between the age groups with respect to seropositivity (P>0.05). Such results do not identify whether horizontal or vertical transmission is the primary route of infection and maintenance of *N. caninum* in beef cattle. Although congenital transmission is considered the most important form of infection for *N. caninum* in cattle, the results of this study suggests that both vertical and horizontal transmission seem to have equal importance in the epidemiology of infection in beef cattle, similar to a study by Moré et al. (2010) in Argentina.

Several experiments also found no correlation between seropositivity and the variables (FERNÁNDEZ; GARCÍA, 2014; GHAREKHANI,

2014; SILVA et al., 2015). Portocarrero et al. (2015) found no correlation between seropositivity and the age or origin (P>0.05) of animals in Peru. Marques et al. (2011) tested 159 samples from the northern state of Paraná and reported that the prevalence of *N. caninum* in cows did not increase proportionally with the age of the infected animal (P=0.43).

Despite 67.14% of seropositive animals to *N. caninum* being females, no association was observed between positive serology and sex (P>0.05). This gender difference can be attributed to sampling where of the total (n=507) samples, 292 (57.6%) were females (Table 1). These values are consistent with those reported by Gharekhani et al. (2014), in which they did not observe statistically significant (P>0.5) differences between seropositivity and age, race or sex of animals tested in Iran.

Table 1. Serology (IFAT, ≥1: 100) to *Neospora caninum* in beef cattle in the mountainous region of Santa Catarina, Brazil.

Variables	Categories	Animals		Positive[1]		Positive[2]	
		n	%	n	%	n	%
Municipalities	Anita Garibaldi	14	2.76	0	0	0	0
	Bocaína do Sul	12	2.37	3	25	3	4.29
	Bom Jardim da Serra	25	4.93	4	16	4	5.71
	Bom Retiro	35	6.9	5	14.29	5	7.14
	Campo Belo do Sul	14	2.76	2	14.29	2	2.86
	Capão Alto	38	7.5	4	10.53	4	5.71
	Correia Pinto	36	7.1	8	22.22	8	11.43
	Lages	107	21.1	16	14.95	16	22.86
	Otacílio Costa	18	3.55	3	16.67	3	4.29
	Painel	38	7.5	3	7.90	3	4.29
	Palmeira	15	2.96	1	6.67	1	1.43
	Ponte Alta	5	0.99	0	0	0	0
	Rio Rufino	7	1.38	0	0	0	0
	São Joaquim	85	16.8	11	12.94	11	15.71
	São José do Cerrito	45	8.88	9	20	9	12.86
	Urupema	13	2.56	1	7.69	1	1.43
Sex	Male	215	42.4	23	10.7	23	32.86
	Female	292	57.6	47	16.1	47	67.14
Age (years)	0≤a≤2	68	13.4	6	8.82	6	8.57
	2<a≤4	195	38.5	26	13.33	26	37.14
	4<a≤8	139	27.4	24	17.27	24	34.29
	>8	105	20.7	14	13.33	14	20
Total	-	507	100	70	13.81	70	100

[1] Relation between the total number of positive animals per variable and the total number of animals from each variable.
[2] Relation between the total of positive animals from each variable and the total number of positive animals.

Although no statistical difference occurred between age and sex with seropositivity separately, these two variables (age and sex) appear to act in concert. It can be noted that females had higher prevalence rates in all age groups compared with males, as well as certain constancy of results in all age groups analyzed (Table 2). This may be due to the majority of tested male animals being at a young age, while older animals were more heavily sampled for females. Thus, taking into account the increased risk of exposure to the parasite with increasing age (BARTELS et al., 2006) and the

differing sample size in age groups, it was expected that older females would contain a greater number of positive animals than young males.

There are few studies about seroprevalence of antibodies against *N. caninum*, specifically in beef cattle. Some data in Brazil reports prevalences of 12.6% for cows and 16.7% for fetuses, in the states of Pernambuco and Alagoas (AMARAL et al, 2012.); 14.6% in pregnant and 15.8% in non-pregnant females, in the state of Paraná (MARQUES et al., 2011) and 19.9% in the region of Presidente Prudente in São Paulo (VIANNA et al., 2008). In contrast, Nascimento et al. (2014)

reported seroprevalence of 30.3% in pregnant zebu cows in the state of Paraná, with a rate of vertical transmission of 29% and a 26.25 fold increased risk of vertical transmission in a seropositive compared to a seronegative cow.

Although reproductive rates were not evaluated in this study, infection with *N. caninum* in beef cattle may represent losses. In Brazil, Andreotti et al. (2010) observed higher seroprevalence of *N. caninum* in animals with gestational problems than in cows that had conceived, and reduced extraction rates in seropositive heifers compared to seronegative animals.

Table 2. Seroprevalence of *Neospora caninum* associated with age and sex of beef cattle from the mountainous region of Santa Catarina, Brazil.

Age (years)		Male n	Male %	Female n	Female %
0≤to≤2	*Positive*	3	6.12	3	15.79
	Negative	46	93.88	16	84.21
		49	100	19	100
2<to≤4	*Positive*	17	12.5	9	15.25
	Negative	119	87.5	50	84.75
		136	100	59	100
4<to≤8	*Positive*	3	10.71	21	18.92
	Negative	25	89.29	90	81.08
		28	100	111	100
>8	*Positive*	0	0	14	13.59
	Negative	2	100	89	86.41
		2	100	103	100
Total	*Positive*	23	10.7	47	16.1
	Negative	192	89.3	245	83.,9
Total		**215**	**100**	**292**	**100**

Conclusion

The results of the present study demonstrated wide distribution, with a relatively low seroprevalence of *N. caninum* in beef cattle herds in the mountainous region of Santa Catarina. The prevalence of antibodies against *N. caninum* was 13.81% and, although with no statistical correlation (P>0.05), a higher incidence of seropositive animals was observed among older females.

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